

**LUNG FUNCTION IS ABNORMAL IN 3 MONTH OLD INFANTS WITH CYSTIC  
FIBROSIS DIAGNOSED BY NEWBORN SCREENING**

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**Online Data Supplement**

## **1. Background**

As part of a longitudinal research programme of infants with CF diagnosed by newborn screening (NBS), this study measured lung function at 3 months in NBS infants with CF and contemporaneous healthy controls of similar age (the focus of the current article), with follow up tests at 1 and 2 years of age (to be reported at a later date). Clinical status including use of medications and anthropometry were documented prospectively. This online supplement (OLS) provides additional details regarding recruitment, methods and results, for which there was no space in the main article.

## **2. Subjects and Methods**

Screened infants with CF, referred to 6 tertiary CF centers participating in the London Cystic Fibrosis Collaboration (LCFC) in the Greater London regions, UK, were eligible for recruitment to the study. The LCFC consists of -

- The Respiratory Unit, Great Ormond Street Hospital (GOSH) for Children NHS Foundation Trust, London;
- The Department of Paediatric Respiratory Medicine, Imperial College & Royal Brompton & Harefield Hospitals NHS Trust, London;
- The Department of Paediatric Respiratory Medicine, Barts & The Royal London Hospital, London;
- Queen Mary's Hospital for Children, Epsom & St Helier University Hospitals NHS Trust, Surrey;
- The Department of Paediatric Respiratory Medicine, Kings College Hospital, London;
- The Department of Child Health, Lewisham Healthcare NHS Trust, London.

## **2.1. Recruitment of screened infants with CF**

Infants diagnosed with CF via NBS with an elevated concentration of immunoreactive trypsinogen (IRT) and confirmation of positive CF transmembrane conductance regulator (CFTR) gene mutation profile and/or sweat chloride test

([http://newbornbloodspot.screening.nhs.uk/nat\\_std\\_cf\\_protocol](http://newbornbloodspot.screening.nhs.uk/nat_std_cf_protocol); date accessed: February 2012) referred to the LCFC centers between January 2009 and July 2011, were eligible for recruitment.

Unless there were any special circumstances, parents were invited to participate in the study by their consultants within approximately 4 weeks of diagnosis during a follow-up “Education” visit to the tertiary CF center. The purpose of the study was explained verbally and written information, together with an illustrated leaflet, was given to parents. The family was allowed time to consider the information and ask further questions before giving written consent.

### ***2.1.1. Inclusion criteria for infants with CF***

- Infants diagnosed with CF by NBS within the Greater London catchment area.

### ***2.1.2. Exclusion criteria for infants with CF***

- Infants with CF born <37 weeks gestation
- Severe congenital disorders, cardio-vascular, skeletal, neuro-muscular or metabolic co-morbidities that could impact on the respiratory system;
- Inability of parents to understand and give informed consent;
- Recruitment contra-indicated on psycho-social grounds;
- History of apnoeic episodes or upper airway pathology;
- Family due to move out of area.

## **2.2. Recruitment of healthy control infants**

Healthy infants of similar age who met the inclusion criteria (see below) were identified monthly using the birth register from the Homerton University Hospital in East London, UK. Since the majority of mothers with babies were discharged from hospital within 24-48 hours following delivery, their family doctors were contacted by post to check that there were no medical and/or social contra-indications for contacting the families in the community. Once confirmation was received from the family doctors, a postal invitation letter, together with a parental information sheet and leaflet describing the lung function tests, were sent to the appropriate families. A phone call was made 7-10 days afterwards to further explain and discuss the study and answer any questions the parents may have with respect to participation.

### ***2.2.1. Inclusion criteria for healthy controls***

- Healthy infants with no congenital abnormalities, born  $\geq 37$  weeks gestation at the Homerton University Hospital, East London;
- Families living within reasonable travelling distance of the Infant Lung Function Laboratory at Great Ormond Street Hospital / UCL Institute of Child Health, and
- Parental consent to lung function measurements under chloral sedation.

### ***2.2.2. Exclusion criteria for healthy controls***

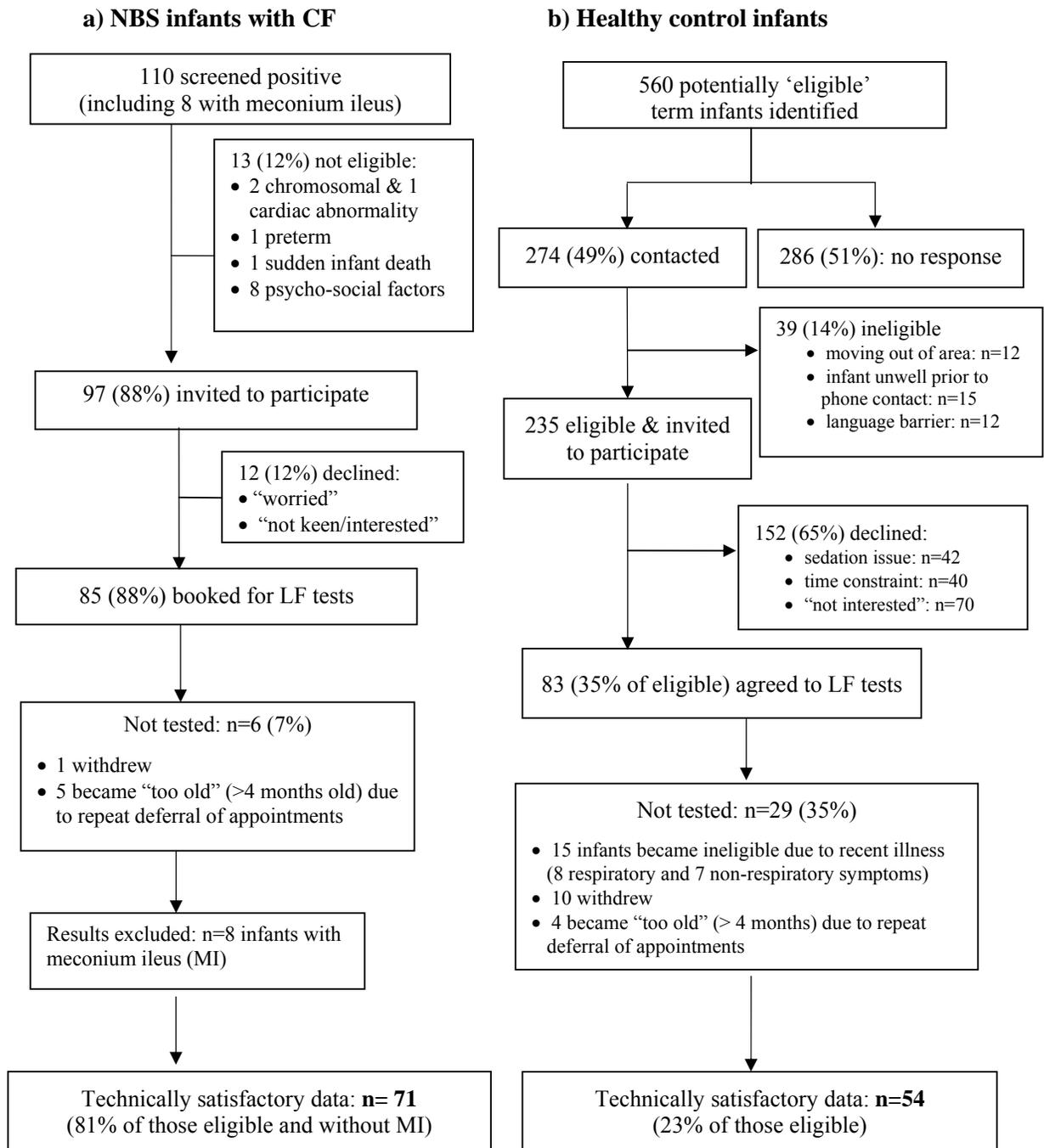
#### **3. Infants born $<37$ weeks gestation**

- Inability of parents to understand and give informed consent;
- Recruitment contra-indicated on medical and/or psycho-social grounds;
- History of apneic episodes or upper airway pathology;

- History of chronic diarrhoea or failure to thrive;
- History of neonatal lung disease, assisted ventilation or co-existent cardio-vascular, skeletal, neuro-muscular, renal or metabolic disorders that could impact on the respiratory system and
- Previous physician diagnosed or hospital admission for lower respiratory tract infections.

Any healthy infant who was recruited to the study but was subsequently admitted to hospital due to respiratory infection, upper airway pathology or who developed chronic diarrhoea or failure to thrive was excluded from the study.

**Figure E1. Flow diagram showing additional details of recruitment process**



**Footnote:** NBS=newborn screen; CF=cystic fibrosis; LF = lung function.

### 3. Data collection

All lung function tests were performed between January 2009 and October 2011 in a single infant lung function laboratory at the Great Ormond Street Hospital / UCL Institute of Child Health, London. Weight and crown-heel length, measured using an infant stadiometer, were expressed as z (or SD) scores to adjust for age and sex.<sup>E1</sup>

#### 3.1. Multiple breath inert gas washout (MBW) technique

Lung clearance index (LCI) assesses overall efficiency of ventilation distribution or gas mixing within the lung and provides a measure of early lung disease; functional residual capacity measured using MBW ( $FRC_{MBW}$ ) represents the resting lung volume that is communicating with the airway opening at time of measurement. The equipment and test procedure for performing MBW in infants were similar to those for preschool children, as described in detail previously (online supplement:

<http://ajrccm.atsjournals.org/cgi/data/171/3/249/DC1/1>)<sup>E2-4</sup> and summarised below.

Data collection was performed in two stages:

- i) the wash-in phase involved the infant inspiring a bias flow of dry air mixture containing the tracer gas (4% sulfur hexafluoride ( $SF_6$ )), 21% oxygen and balance nitrogen, and continued until inspiratory and expiratory  $SF_6$  concentrations were stable and equal at 4% for a minimum of 5-8 breaths, at which time the bias flow was removed;
- ii) the wash-out phase began with the infant inhaling room air and continued until end tidal  $SF_6$  concentration was consistently below 0.1%, i.e., less than  $1/40^{\text{th}}$  of the starting concentration.

LCI is defined as the number of lung volume turnovers (or number of FRCs) required to clear the lungs of the inert tracer gas to  $1/40^{\text{th}}$  of the starting concentration of the tracer gas. Data were acceptable only if there was no evidence of mask leak or flow through the pneumotachometer (PNT).<sup>E5</sup> LCI and  $FRC_{\text{MBW}}$  were calculated as described previously<sup>E2,E4,E6,E7</sup> and reported as the mean of three technically satisfactory MBW recordings. In exceptional cases, the mean of two technically acceptable recordings was used if results were within 5% of one another. During a recent extensive investigation, the CV (SD) for LCI was found to be 4.1 (2.4)% in healthy infants and children, and 8.9 (1.9)% in those with CF (Robinson, P et al, Pediatric Pulmonology, in press; ‘Abbreviated multi-breath washout for calculation of Lung Clearance Index’). For the current study, only 4% of MBW washouts failed to meet quality control criteria.

### ***3.1.1. Prediction equations for lung clearance index and $FRC_{\text{MBW}}$***

Until recently, there were no published reference equations for LCI data during infancy, results being reported as absolute values with a fixed upper threshold to identify abnormally elevated results,<sup>E3,E8</sup> despite the fact that somewhat elevated values have been noted in healthy infants when compared with older children.<sup>E9</sup> Recent collation of MBW data from 497 subjects (48% boys) on 659 test occasions from birth to 19 years of age collected using identical methods and equipment from 3 centres (London, Sweden and Toronto) has now allowed appropriate reference equations to be developed for both LCI and  $FRC_{\text{MBW}}$ .<sup>E10</sup> These reference equations were constructed using the LMS [Lambda (L), Mu (M), Sigma (S)] method as described previously.<sup>E11, E12</sup> Together the L, M and S coefficients are combined algebraically to convert an individual observation to a z-score.

$$\text{z-score} = [(\text{Measurement}/M)^L - 1] / [L*S]$$

$$\text{Upper Limit of Normal (ULN, i.e. 97.5}^{\text{th}} \text{ percentile)} = M*(1.96*S*L + 1)^{1/L}$$

After adjusting for length or height, age and sex did not contribute to the model when predicting LCI whereas for  $FRC_{MBW}$ , length, age and sex all made a significant contribution.

*Reference equation for LCI*

**Table E1: Paediatric reference equations for LCI and  $FRC_{MBW}$**

<b>LCI</b>		
<b>L</b>		-0.81
<b>M (Predicted)</b>		$5.99 + (73.86 * \text{height}^{-1})$
<b>S</b>		0.08
<b>ULN</b>		$\text{Predicted} * ((1.96 * 0.08 * -0.81) + 1)^{1/-0.81}$
<b>FRC</b>		
<b>L</b>		0.19
<b>M (Predicted)</b>		$\exp(-11.10 + (2.12 * \ln \text{Height}) + (0.27 * \text{age}^{0.5}) + (0.04 * \text{sex}))$
<b>S</b>		$\exp(-1.67 + 148.57 * \text{height}^{-2})$
<b>ULN</b>		$\text{Predicted} * ((1.96 * S * 0.19) + 1)^{1/0.19}$

*Footnote: Based on these equations, the ULN for LCI can be simplified to Predicted LCI \* 1.18; whereas that for  $FRC_{MBW}$  becomes  $((0.36 * S + 1)$  to the power 5.37) \* Predicted FRC.*

Results from this study have been expressed as SD- or z-scores according to these equations, with LCI also being expressed in absolute values where appropriate to facilitate comparison with previous publications (Table E2). A comparison of LCI and  $FRC_{MBW}$  z-scores, as well as the difference between plethysmographic and MBW z-scores for FRC ( $\Delta FRC$ ), in infants with CF versus healthy controls is presented in the main article (Table 3, Figure 3).

### 3.2. Plethysmographic FRC and Tidal Breathing variables assessed using the Jaeger MasterScreen BabyBody system (v4.65; CareFusion)

Measurements of plethysmographic FRC were obtained in accordance with ATS/ERS guidelines.<sup>E13</sup> After several regular breaths had been recorded, a brief airway occlusion was performed to ensure that there was an airtight seal around the face mask prior to lowering the hood to close the plethysmograph. **Tidal breathing variables** (tidal volume ( $V_T$ ); respiratory rate (RR); minute ventilation ( $V_E$ ) and the ratio of time to reach peak expiratory flow/expiratory time ( $t_{PTEF/t_E}$ ) were recorded for 2-3 minutes while the plethysmograph reached thermal equilibrium. Full details for Jaeger infant box calibration and FRC data collection have been reported previously.<sup>E14</sup>

The criteria for technical acceptability when collecting  $FRC_{pleth}$  data were:

- no evidence of mask leak (no flow through the PNT and no decay of pressure plateau at the airway opening during the occlusion), with the pre-occlusion end expiratory level from the time-based tidal volume trace being re-established within 5–10 breaths post-occlusion;
- ideally, three (minimum two) complete respiratory efforts against the occlusion were recorded. This is essential for adequate correction of the box volume drift, the magnitude and direction of which will change during the airway occlusion, despite establishing a stable trace prior to the occlusion;
- changes in plethysmographic pressure in phase with changes in pressure at the airway opening during airway occlusion, with no evidence of phase lag or “looping” due to glottis closure, leak or poor drift correction.

At least five satisfactory occlusion maneuvers were performed for each infant. The mean of 3–5 measures of  $FRC_{pleth}$  (minimum 2 if highly repeatable and of good quality) that were within 10% were reported.

### **3.3. Total respiratory compliance ( $C_{rs}$ ) and resistance ( $R_{rs}$ )**

Passive respiratory mechanics ( $C_{rs}$  and  $R_{rs}$ ) was assessed using the single occlusion (SO) technique via the Resistance/Compliance Program of the Jaeger MasterScreen BabyBody system (v 4.65, CareFusion, USA). Details of the protocol for data collection have been described previously.<sup>E15</sup> After 5–10 regular breaths had been recorded to establish a stable end-established level (EEL), a brief airway occlusion was performed at the end of a tidal inspiration, temporarily maintaining lung volume above the EEL to evoke the Hering Breuer Inflation reflex and hence, respiratory muscle relaxation.<sup>E15,16</sup> During airway occlusion (i.e., a constant lung volume with no flow), rapid equilibration occurs within the lungs. The pressure plateau recorded at the airway opening ( $P_{ao}$ ) during such occlusions represents the alveolar pressure, which in turn represents the summed elastic recoil pressure of the lung and chest wall.  $C_{rs}$  is calculated as the volume above the relaxed EEL divided by  $P_{ao}$ . Provided expiration remains relaxed following release of airway occlusion and the respiratory system can be described by a single time constant, the slope of the relaxed expiratory portion of the flow-volume curve represents the expiratory time constant of the respiratory system ( $t_{rs}$ ). Since  $t_{rs}$  is the product of  $C_{rs}$  and  $R_{rs}$ , resistance can be calculated as  $t_{rs}/C_{rs}$  minus the resistance of the apparatus.<sup>E16</sup>

Criteria for technically satisfactory data were:

- Smooth expiration, proceeding to within 10% of previous expiration with no evidence of glottic closure, braking or active expiratory efforts;

- Duration of the pressure plateau at the airway opening  $\geq 100$  ms with variability  $< 10$  Pa;
- Linearity of the flow-volume curve over at least 40% of expiration with  $r^2 > 0.99$ .

$C_{rs}$  and  $R_{rs}$  results were reported using the mean from 3–5 technically acceptable maneuvers. For the calculation of predicted values and z-scores for  $FRC_{pleth}$ , tidal volume ( $V_T$ ), respiratory rate (RR), the ratio of time taken to reach peak tidal expiratory flow: total expiratory time ( $t_{PTEF} : t_E$ ), passive respiratory mechanics ( $C_{rs}$  and  $R_{rs}$ ), please refer to recently created reference equations derived from healthy infants studied with identical equipment and protocols.<sup>E17</sup>

### **3.4. Raised volume (RV) forced expiratory maneuvers**

Prior to performing the raised volume forced expiratory maneuvers, tidal forced expiratory maneuvers<sup>E18</sup> were undertaken to determine the optimal jacket compression pressure ( $P_j$ ) at which flow limitation was achieved, i.e., the point at which no further increase in expiratory flow was observed despite further increases in applied jacket pressure.<sup>E18,19</sup> The optimal  $P_j$  thus obtained was used for the raised volume maneuvers to obtain “full” or raised volume forced expiratory variables.

Airway function at raised lung volume was assessed as previously described.<sup>E20,21</sup> Expiration was forced from an augmented lung volume using an inflation pressure of 30 cmH<sub>2</sub>O (2.93kPa) and maneuvers repeated until three (minimum of two) technically acceptable and reproducible (sum of  $FEV_{0.5}$  and FVC within 10% of each other) flow-volume curves were obtained.  $FEV_{0.5}$ , FVC and  $FEF_{75}$  and  $FEF_{25-75}$  were reported from the “best” curve, defined as the technically satisfactory maneuver with the highest sum of  $FEV_{0.5}$  and FVC.<sup>E20</sup> The criteria for technically acceptable forced expired flow-volume curves were: peak expiratory

flow achieved prior to 10% of expired volume, complete expiration towards residual volume (RV) (i.e., no evidence of early inspiration) and no marked flow transients or glottic closure.<sup>E20</sup> In our laboratory, following quality control, the coefficient of variability for FEV<sub>0.5</sub> during the first year of life is 3.6% (95% CI: 3.1%; 4.1%). Results were expressed as z-scores to account for sex, age and/or body length, adjusted for the equipment used (Jaeger Masterscreen BabyBody) as described previously.<sup>E22</sup> Reduced airway function was defined as a z-score below -1.96 (<2.5<sup>th</sup> centile).

#### **4. Lung Function Results**

Table E2 summarises lung function results in absolute values and after adjustment for body weight at the time of tests for both the CF and control groups to allow for comparisons with the previous literature. For comparison, please refer to Table 4 in the main article, where results are presented more appropriately as z-scores, to adjust for sex, age and body size where necessary.

With the exception of a significantly lower FEV<sub>0.5</sub> in those who had received any antibiotics for symptoms or positive cough swab (i.e., in addition to the routine prophylactic Flucloxacillin that was prescribed for all infants), there was no significant association between lung function outcomes and the infants' genotype, clinical status or treatment prior to the lung function tests at 3 months of age.

Table E3 compares mean z-scores between subgroups with and without homozygous  $\Delta$ F508, respiratory symptoms, positive culture on cough swab and use of additional antibiotic.

Differences are presented with 95% confidence intervals together with p-values from two sample *t*-tests. FEV<sub>0.5</sub> z-score was significantly lower in those who had received additional antibiotics (difference: -0.7 (-1.29, -0.1), p=0.049). All other differences were non-

significant. However, note that some confidence intervals were quite wide and we could not exclude some differences of clinical importance. This sub-group analysis is exploratory.

**Table E2: Comparison of lung function (absolute values and in relation to weight at test) in infants with CF and healthy controls**

		Infants with CF n=71		Healthy controls n=54	$\Delta$ (95% CI) CF- controls	p values
<b>Weight at test</b>		5.32 (0.84)		6.05 (0.78)	-0.73 (-1.02; -0.45)	<0.001
<b>Weight z-score</b>		-0.59 (1.1)		0.25 (1.04)	-0.84 (-1.22; -0.46)	<0.001
<b>Lung Function results</b>						
<b>Multiple breath washout</b>	n		n			
LCI	70	7.89 (0.92)	51	7.46 (0.55)	0.46 (0.19; 0.72)	0.002
FRC <sub>MBW</sub> , mL	70	105.7 (22.0)	51	106.7 (19.8)	-1.2 (-8.8; 6.5)	0.800
FRC <sub>MBW</sub> , mL/kg	70	20.0 (3.4)	51	18.0 (3.5)	2.0 (0.8; 3.3)	0.002
<b>Tidal breathing</b>						
Respiratory rate, per min	71	37.8 (7.6)	54	36.5 (6.5)	1.3 (-1.2; 3.8)	0.297
Tidal volume, mL	71	51.4 (8.1)	54	54.6 (7.0)	-3.2 (-5.9; -0.6)	0.018
Tidal volume, mL/kg	71	9.7 (1.3)	54	9.1 (1.2)	0.6 (0.2; 1.1)	0.007
t <sub>PTEF</sub> /t <sub>E</sub> , %	71	28.7 (10.1)	54	30.3 (9.4)	-1.6 (-5.1; 1.8)	0.356
Minute ventilation, mL/min	71	1913 (342)	54	1968 (285)	-55 (-166; 57)	0.332
Minute ventilation, mL/min/kg	71	363 (61.1)	54	329 (47.9)	35 (15; 54)	0.001
<b>Passive mechanics</b>						
C <sub>rs</sub> , mL/kPa	47	67.3 (13.5)	41	73.9 (10.7)	-6.7 (-11.8; -1.5)	0.011
C <sub>rs</sub> , mL/kPa/kg	47	12.7 (1.9)	41	12.3 (1.8)	0.38 (-0.40; 1.16)	0.331
R <sub>rs</sub> , kPa/L/s	47	5.50 (1.41)	41	4.83 (1.28)	0.63 (0.06; 1.20)	0.031
<b>Plethysmography</b>						
FRC <sub>pleth</sub> , mL	56	111.3 (24.7)	47	104.4 (21.2)	7.0 (-2.0; 15.9)	0.127
FRC <sub>pleth</sub> , mL/kg	56	20.9 (4.0)	47	17.3 (3.5)	3.6 (2.1; 5.0)	<0.001
$\Delta$ FRC (pleth-MBW), mL/kg	55	1.1 (4.1)	45	-0.4 (3.2)	1.5 (0.05; 2.9)	0.043
<b>Raised volume technique</b>						
FEV <sub>0.5</sub> , mL	68	146.2 (27.1)	52	177.3 (25.2)	-31.0 (-40.5; -21.5)	<0.001
FVC, mL	68	172.8 (34.3)	52	208.5 (31.0)	-35.7 (-47.6; -23.9)	<0.001
FEF <sub>25-75</sub> , mL/s	68	316.0 (87.0)	52	380.2 (85.5)	-64.2 (-95.7; -32.8)	<0.001
FEF <sub>75</sub> , mL/s	68	169.6 (55.5)	52	207.1 (57.2)	-37.5 (-58.2; -16.8)	<0.001

Data expressed as mean (SD)

$\Delta$  = mean difference between groups; CI = confidence interval of the difference.

**Table E3. Associations between potential clinical determinants and lung function outcomes at 3 months of age in infants with CF**

Predictor	Outcome			
	Homozygous $\Delta F508$ (n=42/71)	Respiratory symptoms (cough and/or wheeze), ever <sup>†</sup> (n=43/71)	Positive growth on cough swab, ever <sup>†</sup> (n= 16/71)	Received additional antibiotics, included nebulised & intravenous <sup>†</sup> (n=52/71)
LCI z-score	0.04 (-0.71; 0.78) p = 0.921	0.02 (-1.65; 0.68) p = 0.964	-0.40 (-1.22; 0.42) p = 0.335	0.37 (-0.33; 1.06) p = 0.092
FEV <sub>0.5</sub> z-score	-0.14 (-0.80; 0.53) p = 0.683	-0.52 (-1.09; 0.54) p = 0.075	-0.06 (-0.76; 0.65) p = 0.878	-0.70 (-1.29; -0.10) p = 0.049
FEF <sub>25-75</sub> z-score	-0.03 (-0.79; 0.74) p = 0.947	-0.25 (-0.92; 0.42) p = 0.458	-0.32 (-1.15; 0.51) p = 0.444	-0.61 (-1.30; 0.09) p = 0.306
FRC <sub>pleth</sub> z-score	0.27 (-0.43; 0.97) p = 0.443	-0.14 (-0.76; 0.47) p = 0.640	0.08 (-0.68; 0.85) p = 0.829	0.06 (-0.60; 0.71) p = 0.860

**Footnote:** See Figures 1 and 2 in main article for further details.

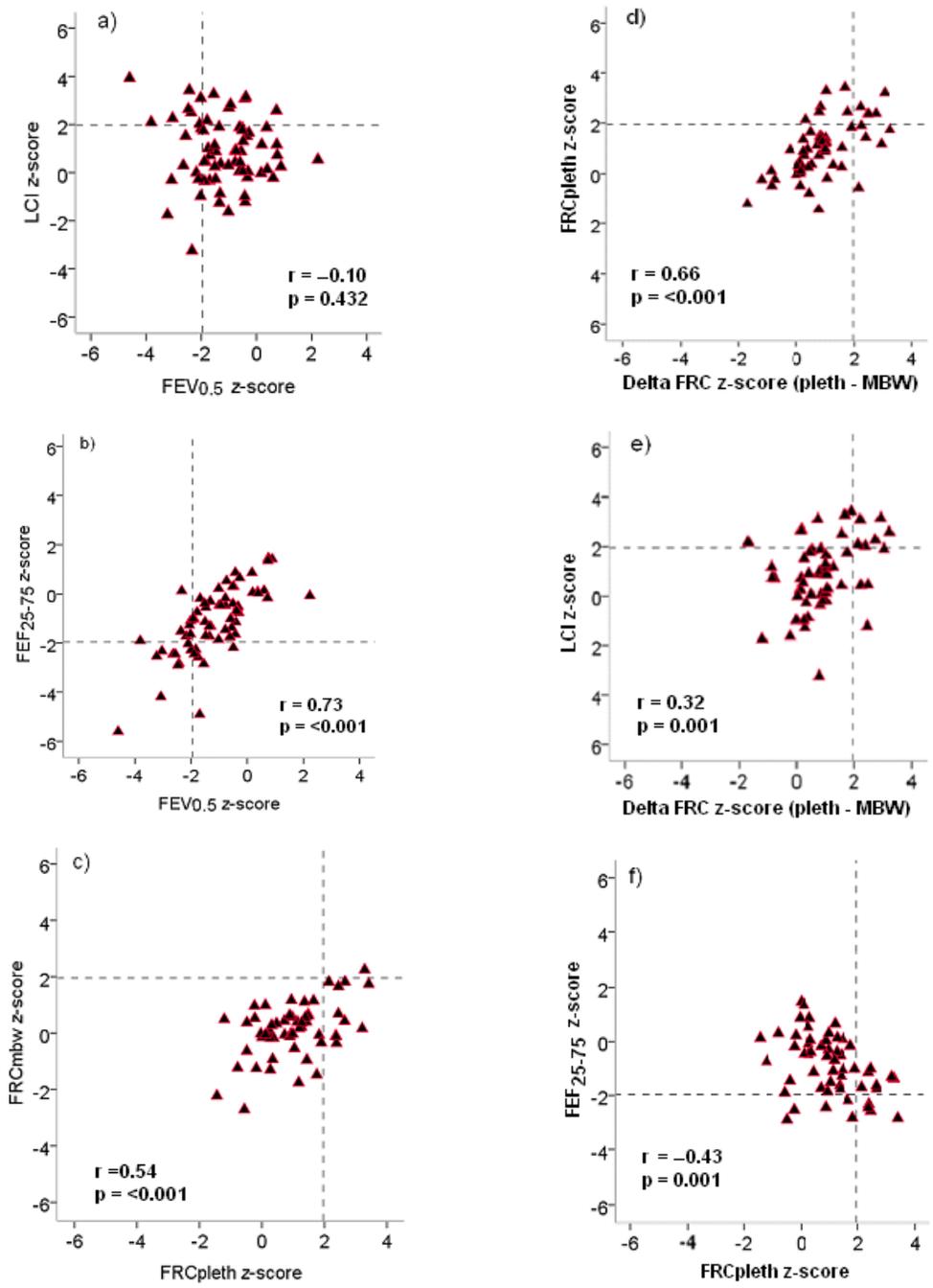
LCI: lung clearance index; FEV<sub>0.5</sub>: forced expired volume in 0.5s; FEF<sub>25-75</sub>: forced expired flow between 25–75% of forced vital capacity; FRC<sub>pleth</sub>: plethysmographic functional residual capacity.

<sup>†</sup> duration between diagnosis of CF to time of lung function tests

#### 4.1. Associations between different lung function outcomes

For clarity, the following paragraph is reproduced here from the main article in order to explain the graphs in Figure E2, which shows the relationship between selected pulmonary function outcomes in infants with CF. As can be seen, Figure E2a illustrates that there was no significant relationship between the two primary outcome measures, FEV<sub>0.5</sub> and LCI ( $r = -0.16$ ,  $p=0.09$ ), suggesting that they reflect different aspects of underlying pathophysiology. The RV forced expiratory technique is likely to be most sensitive in early life when the chest wall is highly compliant and airway closure more likely to occur even in the tidal range. Airflow limitation is thus more likely to occur in the presence of relatively mild lung disease than in later life. This situation changes by the preschool years, when spirometry becomes less discriminative in CF lung disease than during infancy despite overall disease progression,<sup>E23</sup> such that by 4 years of age the LCI is by far the most discriminative test.<sup>E24</sup> During early infancy, mild airway obstruction in CF may be associated with less uneven distribution of ventilation than in later life, and hence less impact on LCI. In addition, the increased LCI found among younger subjects due to developmental differences<sup>E9</sup> may make LCI less discriminatory during early life. 21% infants had an LCI above 1.96 z-scores, whereas 25% had an FEV<sub>0.5</sub> below -1.96 z-scores. If using either test, 35% (24/68) would be identified with abnormal results, whereas only 12% (8/68) had abnormalities detected by both these tests. Both FEV<sub>0.5</sub> and FEF<sub>25-75</sub> ( $r = 0.73$ ,  $p<0.0001$ ) detected a similar proportion of infants outside the normal range (25% and 24% respectively), whereas FEF<sub>75</sub> was less discriminative (only detecting abnormalities in 15% infants) (Figure E2b). FRC<sub>pleth</sub> z-score and delta FRC were highly correlated ( $r = 0.66$ ,  $P<0.001$ ) (Figure E2d) and both detected a similar proportion of infants with abnormally elevated results (18% and 20%, respectively). Forty four percent (31 /71) NBS CF infants had at least one abnormal result if based on MBW, plethysmography or the raised volume technique.

**Figure E2. Association between selected pulmonary function outcomes in infants with CF**



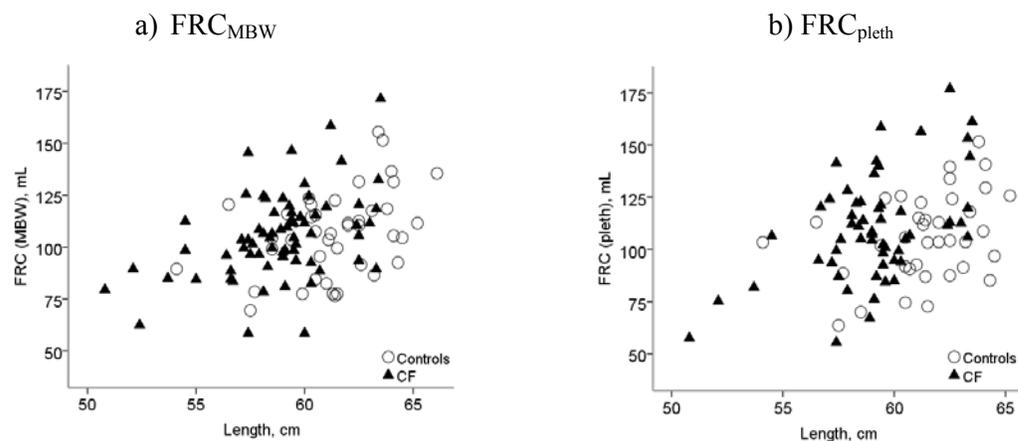
**Legend:** The horizontal dashed line denotes *either* the upper limit of normality for LCI and FRC (1.96 z-score) *or* the lower limit of normality (-1.96 z-score) for FEF<sub>25-75</sub>; the vertical dashed line represents *either* the upper limit of normality for FRC (1.96 z-score) *or* the lower limit of normality (-1.96 z-score) for FEV<sub>0.5</sub>.

## 4.2. Impact of CF on lung function

### 4.2.1. Lung volumes

The relationship between  $FRC_{MBW}$  and  $FRC_{pleth}$  and body length in infants with CF and healthy controls is shown in Figure E3. It can be seen that despite being of similar age, CF infants tended to be shorter at time of test, and that despite much overlap,  $FRC_{MBW}$  was slightly higher at any given length in those with CF (Figure E3a, Table E2) as reflected by the small, but significant increase in  $FRC_{MBW}$ , once differences in length are adjusted for by expressing results as z-scores (Table 3, main article). This difference would, however, have been over-estimated had results simply been expressed as a ratio of body weight (Table E2), due to the relative growth restriction in those with CF by 3 months.

**Figure E3. FRC measured using the MBW and plethysmograph respectively as a function of crown-heel length at time of test**



When  $FRC_{pleth}$  was plotted versus body length, despite considerable overlap between the groups, relatively higher values were observed in infants with CF when compared with controls of similar body size. Thus, although there was no significant difference between the groups when results were expressed in absolute terms (Table E2), after adjusting for length by

expressing results as z-scores,  $FRC_{\text{pleth}}$  was significantly higher in those with CF (Table 3 and Figure 3c in main article). Again, had results simply been expressed as a ratio of body weight (Table E2), this difference would have been over-estimated.

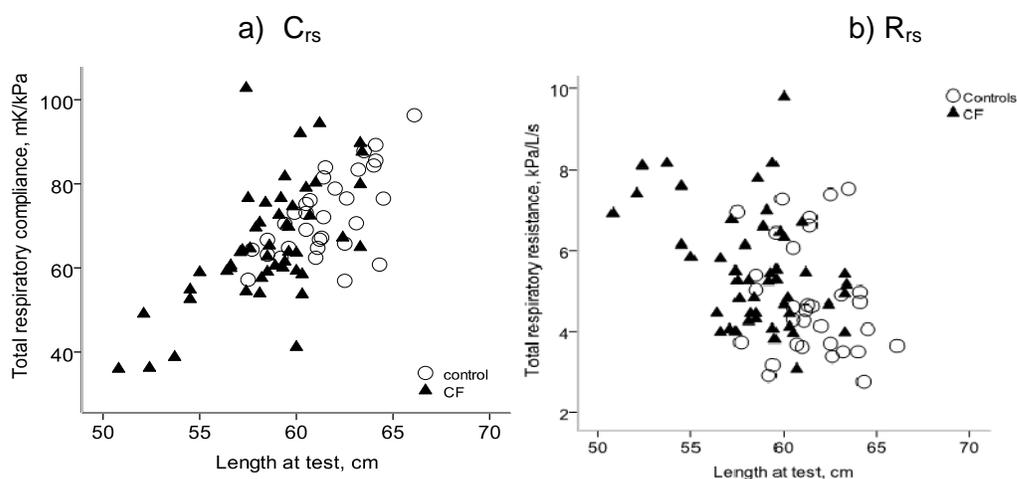
#### **4.2.2. Effect of CF on tidal breathing variables**

There was no difference in respiratory rate or  $t_{\text{PTEF}}/t_{\text{E}}$  between infants with CF and healthy controls, whether results were expressed in absolute terms or as z-scores (Table E2, and Table 3 in main article). While tidal volume appeared lower in those with CF when expressed in absolute terms (Table E2), once expressed as z-scores to correct for body size and age (section 3.4.1), tidal volume was significantly higher in those with CF (Table 3, main article). Tidal volume was also increased in CF if results were expressed in mL/kg body weight, although this approach is not recommended as it will tend to over-estimate differences in growth restricted infants.<sup>E17</sup>

#### **4.2.3. Effect of CF on Respiratory mechanics**

The relationship between respiratory mechanics and length according to diagnostic group is illustrated in Figure E4. It can be seen that while values of  $C_{\text{rs}}$  were lower and  $R_{\text{rs}}$  higher in those with CF when expressed as absolute values (Table E2), this was largely related to body size at time of test;  $C_{\text{rs}}$  increasing and  $R_{\text{rs}}$  decreasing with somatic growth. After adjusting for length and/or age by expressing results as z-scores,<sup>E17</sup> no significant difference was observed between the groups with respect to either  $C_{\text{rs}}$  or  $R_{\text{rs}}$  (Table 3 and Figure 3b in main article).

**Figure E4. Comparison of: a)  $C_{rs}$  and b)  $R_{rs}$  in NBS CF infants and healthy controls at 3 months of age**



#### **4.2.4. Effect of CF on forced expired flows and volume**

As can be seen from Table E2, there were highly significant reductions in FVC, forced expired volume and flows among infants with CF at 3 months when results were expressed in absolute terms ( $p < 0.001$  for all). However, given the shorter length for age amongst those with CF, a more accurate estimation of this reduction is obtained once results are expressed as z-scores to adjust for sex and body size, as presented in Table 3 of the main article.

### **5. Validation of parental report of smoking exposure**

In this study, the reported incidence of maternal smoking during pregnancy and postnatally were relatively low ( $\leq 13\%$ ; Table 1, main article), when compared with values from infants in London reported a decade ago.<sup>E25</sup> Table E4 summarises the level of cotinine concentrations (a metabolite of nicotine) for infant urine and maternal saliva, collected from those whose mothers reported no smoking during pregnancy and postnatally. The results were well below the reported optimum cut-off values to distinguish non-smokers from smokers: i.e., 50 ng/mL

for urine and 12 ng/mL for salivary samples,<sup>E26</sup> inferring that parents in this cohort were honest when reporting their smoking habit, thus suggesting that passive smoke exposure is not likely to bias interpretation of infant lung function in this study.

**Table E4. Urine and salivary cotinine results**

	n	Infants with CF	n	Healthy controls	$\Delta$ (95% CI) CF- controls
non-smoking mothers	63		50		
Infant urine cotinine, ng/mL	45	1.1 (0.4) (range: 0.1 –3.0)	26	2.8 (5.4) (range: 0.9–24.5)	-1.8 (-4.0; 0.4) p =0.148
Maternal saliva cotinine, ng/mL	8	0.1 (0.01) (range: 0.1 – 0.1)	11	0.9 (2.2) (range: 0.1 – 7.6)	-0.8 (-2.3; 0.7) p =0.276

Data were expressed as mean (SD) unless otherwise stated.

CF= cystic fibrosis; CI=confidence interval.

$\Delta$  = mean difference between groups.

## **6. Standardised treatment for newborn infants with CF**

Prior to commencement of this study, a standardised protocol was developed and agreed upon by all participating consultants. This was adhered to throughout the duration of the study.

Following diagnosis, all infants commenced on multivitamins, pancreatic supplements (if pancreatic insufficient) and prophylactic flucloxacillin (25mg/kg twice daily).<sup>E27</sup> The extent to which protocol was adhered to was checked both by regular review of prospectively completed Case Record Forms (CRF) and by discussions at collaborative meetings of the LCFC. There was no evidence of deviations from this protocol prior to or at the time of the 3 month lung function tests. Cough swabs were taken routinely at clinic visits (minimum every 2–3 monthly) and additionally when the infant is symptomatic. A standardised protocol for collection, storage and analysis of samples was used.<sup>E28</sup>

Within the UK, all centres encourage daily chest physiotherapy to infants and children with CF. Within the London CF Collaboration, parents/carers of CF infants and children are educated about the importance of physical activity and its benefits on the respiratory system, and they are taught an appropriate airway clearance technique. Physiotherapy is carried out as appropriate to the child's condition and reviewed frequently in conjunction with medical treatment.

### **6.1. Infection with *Pseudomonas aeruginosa* (PsA)**

*(a) First growth- Monthly cough swabs were collected while on treatment.*

- **Well infant (based on clinical judgement)**
  - Home therapy with 3 weeks of oral ciprofloxacin (15mg/kg twice daily) and
  - 3 months of nebulised Colistin (Colomycin: 1 million units twice daily).<sup>E29</sup>
- **Unwell infant (based on clinical judgement)**
  - Hospital admission for 2 weeks of intravenous (IV) antibiotics. The choice of antibiotics was guided by results from culture and sensitivity;

- Intravenous Ceftazidime (50mg/kg three times daily) and intravenous Tobramycin (10mg/kg once daily);
- Also started on 3 months of nebulised Colistin, initiated whilst in hospital.

***(b) Re-growth during the initial 3 month treatment period (whilst still on Colistin)***

- **Well infant**
  - Further 3 weeks of oral Ciprofloxacin.
- **Unwell infant**
  - Hospital admission for intravenous antibiotics and a further 3 months of nebulised Colistin. (If second course of intravenous antibiotics was inappropriate, 3 weeks of oral Ciprofloxacin was given instead.)

***(c) Regrowth at the end of 3 weeks ciprofloxacin or 3 months nebulised Colistin***

- **intravenous antibiotics**, and either
  - a further 3 months of nebulised Colistin, or
  - monthly alternating nebulised Colistin and Tobramycin (300mg twice daily).

**6.2. Infection with *Staphylococcal aureus* (SA)**

***(a) First growth***

- **Well infant**
  - Oral Augmentin Duo (400/57) 0.3mL/kg twice daily for 2–4 weeks, *or* an equivalent dose of co-amoxiclav syrup (0.25mL/kg of 250/62 strength) three times daily for 2–4 weeks based on clinical judgment.
- **Unwell infant**
  - Hospital admission for 2 weeks of IV antibiotics;
  - Intravenous Tobramycin once daily and intravenous Teicoplanin 10mg/kg for 2 doses twelve hours apart then 10mg/kg once daily.

***(b) Regrowth less than 6 months from first growth***

- Oral flucloxacillin 50mg/kg for 28 days.

**(c) Further regrowth within 6 months**

- Two oral anti-staphylococcal antibiotics for 4 weeks.

**6.3. Infection with *Haemophilus influenzae* (HI)**

**(a) First growth**

- **Well infant**

- Oral Augmentin Duo or co-amoxiclav syrup for 2–4 weeks (based on clinical judgement)

- **Unwell infant**

- Hospital admission for 2 weeks of intravenous antibiotics

**(b) Regrowth less than 6 months from first growth**

- Oral Augmentin Duo or co-amoxiclav syrup for 2–4 weeks (based on clinical judgement)

**(c) Further regrowth within 6 months**

- Clarithromycin (7.5mg/kg twice daily) for 2–4 weeks

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